Onset of Type 1 Diabetes Mellitus in Infancy after Enterovirus Infections

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Enterovirus infections may initiate and accelerate the beta-cell damaging process leading to Type 1 (insulin-dependent) diabetes mellitus (Type 1 DM). Recent prospective studies have suggested that this can happen long before overt disease and even *in utero*. We describe an infant, followed regularly from birth, who progressed to clinical Type 1 DM at the age of 14 months. He had a strong enterovirus exposure exceptionally early in life; the first enterovirus infection occurred before the age of 3 months and the second between the age of 9 and 12 months. The first infection probably occurred at birth, when the child had symptoms of a respiratory infection. This infection was followed by the appearance of beta-cell autoimmunity, and clinical Type 1 DM was diagnosed shortly after the second infection. The child had a low level of maternal enterovirus antibodies and short duration of breast-feeding, both associated with increased risk for enterovirus infections during the fetal period and infancy. This case fits with the current hypothesis that enterovirus infections can induce the process resulting in Type 1 DM, especially when occuring early in life. Furthermore, this demonstrates the feasibility of the present study design, which is applicable also in large-scale birth-cohort studies. © **1998 John Wiley & Sons, Ltd.**

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Introduction

Recent prospective studies have suggested that enterovirus infections may initiate beta-cell destruction resulting in Type 1 (insulin-dependent) diabetes mellitus (Type 1 DM).[1-4] This can happen several years before clinical diabetes appears and be related not only to childhood infections[1,4] but also to maternal infections during pregnancy which increase the risk of diabetes in the offspring.[1-3] The timing of the infection may be crucial and the risk may be highest when the child is exposed *in utero* or early infancy, when the immune system is immature and elimination of virus ineffective. This is reflected by the occurrence of severe life-threatening enterovirus infections in newborn infants which are rare in older children.[5] Moreover, certain other viruses, like hepatitis B viruses, are known to establish chronic infections when infecting fetuses or newborn infants.

Prospective follow-up studies evaluating the possible diabetogenic role of enterovirus infections during the first months of life have not been published. We describe here a child who was followed from birth until clinical diagnosis of Type 1 DM at the age of 14 months. Serum samples were taken routinely at the age of 3, 6, 9, 12, and 14 months for enterovirus antibody determinations, as part of an ongoing study. A maternal serum sample taken at the end of the third month of pregnancy was also available for virological studies.

Case Report

The male infant was born 4 weeks prematurely by a caesarean section in August 1992 at the 2nd Department of Obstetrics and Gynaecology, University of Helsinki. The birth weight was 3.245 kg and length 48 cm. He carried the HLA-DQB1 allele 0302 but none of the protective alleles 0602, 0603, or 0301 (for methods see Sjoroos *et al.*[6]), indicating an increased genetic susceptibility for Type 1 DM.

The infant was suspected of having a respiratory infection or respiratory distress syndrome at birth, since

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he had decreased O_2 saturation (<90%) and pulmonary congestion and his C-reactive protein (CRP) concentration increased from 18 to 38 mg l^{-1} on the second day after birth. After 4 days of treatment with additional oxygen, an antibiotic and a diuretic, he was clinically well and his CRP concentration had returned to normal values. Blood culture gave a negative result.

The infant had high levels of both IgM and IgG class enterovirus antibodies in the first serum sample taken at the age of 3 months, indicating an enterovirus infection during the first months of life (for methods see Hyoty *et al.*[1], Hiltunen *et al.*[4]). This infection may have been responsible for the respiratory symptoms observed at birth. The infant had also a second enterovirus infection between 9 and 12 months of age, indicated by rises in IgM and IgG class antibodies (Figure 1(a)).

The first sign of beta-cell autoimmunity appeared soon after the first enterovirus infection: insulin autoantibodies (IAA) between 3 and 6 months of age and islet cell antibodies (ICA) and antibodies to the 65 kD isoform of glutamic acid decarboxylase (GAD65A) between 6 and 9 months of age (Figure 1(b)); Antibodies against IA-2 (IA-2A) appeared within the same time interval as the second enterovirus infection, i.e. between 9 and 12 months of age (Figure 1(b); for autoantibody methods see Kulmala *et al.*[7]) Clinical Type 1 DM was diagnosed at the age of 14 months.

We analysed maternal enterovirus antibody levels in the serum sample taken at the end of the third month of pregnancy and recorded the duration of breast-feeding, since maternal antibodies acquired through placenta or in breast-milk offer protection against enterovirus infections in infancy. The mother of this infant had a very low IgG class antibody level against an enterovirus peptide antigen, at 9 EIU, below the cut-off limit for seropositivity (10 EIU). The enterovirus peptide epitope is a common antigenic determinant for several enteroviruses and can be used in the assessment of immunity against the enterovirus genus in general.[4,8] Moreover, the mother had neutralizing antibodies against only one of the six coxsackievirus B serotypes, which are the enteroviruses most implicated in the pathogenesis of Type 1 DM. Finally, the infant was breast-fed for only 35 days and exclusively breast-fed for only 2 days. This duration of breast-feeding was very short, another risk factor for infantile infections.

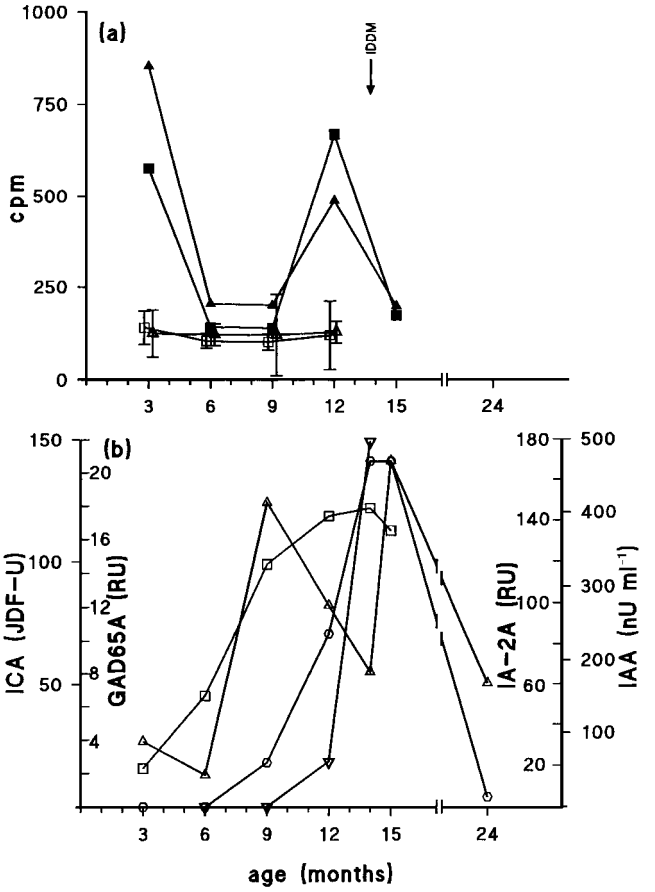

Figure 1. (a) The infant had his first enterovirus infection before the age of 3 months, reflected by high IgM (▲) and IgG (■) class antibody levels against coxsackievirus A9 in the serum sample taken at the age of 3 months. Enterovirus antibody levels were also increased in the serum sample taken at the age of 12 months, indicating another enterovirus infection between 9 and 12 months of age. IDDM was diagnosed at the age of 14 months. The reported case was one of 20 infants taking part in the first pilot study of a Nutritional Prevention of IDDM intervention trial. Median enterovirus antibody levels (±SD) in the other 19 infants are presented as comparison (IgM △; IgG □). (b) Insulin autoantibodies (IAA; □ cut-off limit for positivity 68 nU ml^{-1}) appeared between 3 and 6 months of age, islet cell antibodies (ICA; ⎔ detection limit 2.5 JDF-U) and antibodies to the 65 kD isoform of glutamic acid decarboxylase (GAD65A; △ cut-off limit for positivity 6.6 RU) appeared between 6 and 9 months of age and antibodies against IA-2 (IA-2A; ▽ cut-off limit for positivity 0.43 RU) appeared between 9 and 12 months of age

Discussion

We describe here an infant who presented with Type 1 DM at the age of 14 months, after exposure to enteroviruses early in infancy. The manifestation of clinical diabetes at this age is unusual. For example, in the nationwide Childhood Diabetes in Finland (DiMe) study in the late 1980s, only 1.3% of the 750 cases of Type 1 DM were diagnosed before the age of 15 months (Åkerblom, unpublished observations). Accordingly, if an environmental agent had induced the beta-cell damaging process in this infant, he would have had to have been exposed to this trigger very early in life. The initiating insult must have occurred before the age of 6 months, when the first signs of beta-cell autoimmunity appeared.

The first enterovirus infection, observed before the age of 3 months, could have been the initiating factor, as insulin autoantibodies were detected in the next serum sample. Clinical diabetes manifested only a few months after the second enterovirus infection, which could have had an accelerating or precipitating role in the diabetic process. The temporal relationships between the infections, the autoantibodies and the onset of clinical Type

1 DM give an impression of causality. This impression is supported by large prospective studies, suggesting that enterovirus infections occurring *in utero* or in childhood may initiate the beta-cell damaging process[1–3] and describing a temporal association between enterovirus infections and ICA seroconversion.[4] In this perspective, the observed enterovirus infections may indeed have been causally associated with the beta-cell damage in our infant.

This child was one of 20 infants of mothers with Type 1 DM taking part in the first pilot of a prospective Nutritional Prevention of IDDM intervention trial. This trial was designed to evaluate the possible effect of elimination of cow's milk proteins in early infancy on the risk of manifesting Type 1 DM.[9] The described case was the only infant in this series who progressed to clinical diabetes before the age of 4 years. The potential role of enterovirus infection is supported by the fact that his exposure to these viruses was the strongest observed in the series: he was the only infant with two enterovirus infections during the first year of life and the only infant with evidence of an enterovirus infection occurring before the age of 3 months. In the other 19 infants, only two enterovirus infections were observed: a boy had an infection between 6 and 9 months of age and a girl between 9 and 12 months. The present case belonged to the dietary intervention group receiving a casein hydrolysate formula (Nutramigen, Mead Johnson & Company, Evansville, IN, USA) devoid of intact cow's milk proteins as supplementary milk over his first 9 months of life. This implies that cow's milk proteins were not involved in the induction of the diabetic process and also that the avoidance of cow's milk proteins in infancy does not provide a total protection against Type 1 DM, at least not if a strong enough influence of some other DM-related environmental factor occurs.

It has been suggested that the risk of Type 1 DM can be associated with several enterovirus serotypes and also serotypes other than the most frequently mentioned coxsackievirus B serotypes (Frisk *et al.*[10]; Roivainen *et al.*[11]). The enterovirus infections of the reported case were not caused by any of the coxsackie B serotypes or coxsackievirus A9, as defined by plaque neutralization (data not shown). The RIA-method which was used for the documentation of enterovirus infections was not serotype specific (for method see Hiltunen *et al.*[4]).

The low levels of maternal enterovirus antibodies may have led to poor protection againt enterovirus infections in the infant over his first months of life, as known from other studies.[12] The enterovirus antibody level observed in the mother during pregnancy (9EIU) was much lower than the mean level of 37EIU found in a recent survey of 100 pregnant women in Finland (Lonnrot *et al.*, unpublished observations) or the mean level of 85EIU observed in the other mothers of the Nutritional Prevention of IDDM first pilot trial. Moreover, the mother of this child had neutralizing antibodies against only one out of the six coxsackievirus B serotypes. In the DiMe study, 96 pregnant mothers were studied and neutralizing antibodies were observed against an average of three of the six coxsackievirus B serotypes. The same frequency of neutralizing coxsackievirus B antibodies (against three out of the six serotypes) was observed also in the other 19 mothers of the infants taking part in the pilot study.

Both the duration of total breast-feeding (35 days) and that of exclusive breast-feeding (2 days) were short in the reported case. For example, in the DiMe-study as a whole the mean duration of breast-feeding and exclusive breast-feeding was 196 days and 90 days,[13] respectively, and among the other 19 infants taking part in the pilot study the mean duration was 132 days and 65 days, respectively. The short duration of breast-feeding may have increased the risk of enterovirus infections in this infant, since breast-feeding has been reported to protect against enterovirus infections.[14] Breast-feeding has also been observed to have a protective effect against Type 1 DM,[13] which may be due to the strongly co-associated avoidance of cow's milk proteins in early infancy, but possibly also due to protection against enterovirus infections.

We conclude that the evaluation of various risk factors in the context of time-consuming intervention trials makes these trials more complicated, but may considerably increase their sensitivity to detect possible protective effects of the intervention protocol and can also give information on possible interactions between different environmental factors. This case history demonstrates the feasibility of such a study design and supports the assumption that enterovirus infections during the first months of life may be important inducers of beta-cell damage in genetically susceptible children. Their role should be evaluated in large-scale birth cohort studies.

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